

WE CLAIM:

1. A method for determining the relative timing of the transcriptional activation or repression of genes in a population of stem cells that occurs when the stem cells differentiate, comprising:

(a) randomly inserting into the genomic DNA of stem cells in a population of stem cells a marker DNA construct comprising a nucleotide sequence that encodes a detectable product and is not operably linked to a promoter;

(b) culturing the stem cells ex vivo under conditions in which the stem cells differentiate;

(c) monitoring the differentiating stem cells to detect changes in the level of expression of the marker DNA constructs in the cells; and

(d) detecting differentiating cells in which there is a change in the level of expression of the marker DNA construct, and determining the relative timing of the change in the level of expression of the marker DNA construct that occurs in these cells.

2. The method of claim 1, step further comprising isolating individual stem cells having an inserted marker DNA construct, or colonies of cloned cells derived from such individual stem cells; and then culturing the isolated stem cells in step (b).

3. The method of claim 1, wherein the stem cells are totipotent or nearly totipotent stem cells of a mammal.

4. The method of claim 1, wherein the stem cells embryonic stem cells of a murine, bovine, porcine, non-human primate, or human mammal.

5. The method of claim 1, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and a cell surface protein comprising an antigen that is exposed to the cell exterior.

6. The method of claim 1, further comprising identifying individual stem cells in which there is a change in the level of expression of the marker DNA construct when the stem cells differentiate, and isolating and expanding said stem cells as single cell clones.

7. The method of claim 6, further comprising determining the nucleotide sequence of at least part of the gene in which is inserted a marker DNA construct that changes its level of expression when a stem cell differentiates.

8. The method of claim 7, comprising determining the nucleotide sequence of at least part of the coding sequence or at least part of a transcription-regulating sequence of the gene.

9. The method of claim 1,  
wherein the marker DNA construct encodes a recombinase,  
wherein the same or a different marker DNA construct inserted into the genomic DNA of each stem cell comprises a first nucleotide sequence encoding a detectable product, and a second nucleotide sequence comprising two recombination sites that inhibits the expression of the first nucleotide sequence;  
wherein the recombinase recognizes the two recombination sites, and expression of the nucleotide sequence encoding recombinase results in excision of

the second nucleotide sequence from the stem cell genomic DNA and expression of the first nucleotide sequence encoding the detectable product; and

wherein step (d) comprises determining the relative timing of the transcriptional activation of the marker DNA constructs that occurs when the stem cells differentiate.

10. The method of claim 6, wherein the first nucleotide sequence encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and a cell surface protein comprising an antigen that is exposed to the cell exterior.

11. The method of claim 2, further comprising purifying differentiating cells having a transcriptionally activated marker DNA construct from cells that do not have a transcriptionally activated marker DNA construct, and

using the purified cells or an extract thereof as an immunogen to elicit production of an antibody that binds specifically to a differentiation antigen of the purified cells.

12. The method of claim 11, comprising eliciting production of an antibody that binds specifically to a differentiation antigen on the external surface of the cells.

13. The method of claim 12, comprising eliciting production of a monoclonal antibody that binds specifically to a differentiation antigen on the external surface of the cells.

14. The method of claim 11, wherein the marker DNA construct comprises a nucleotide sequence that encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, an antigenic cell surface protein that is exposed to the cell exterior, or a fusion protein comprising at least one of the foregoing proteins, and the cells are purified by cell sorting, laser dissection, or immunoaffinity separation.

15. The method of claim 11, wherein the marker DNA construct comprises a nucleotide sequence encoding a protein that confers resistance to a selection agent, and purification of the cells comprises culturing the cells in the presence of an agent that selects against non-resistant cells.

16. The method of claim 1 which includes a secondary screening step to identify multiple genes that are transcriptionally activated in cells that differentiate into a particular cell phenotype.

17. The method of claim 16 wherein screening is effected using an hybrid probe, an antibody, or a 2-D gel.

18. A method for identifying genes in a cell that are transcriptionally activated at different times during differentiation of a stem cell into the particular cell type, comprising:

(a) identifying genes that are transcriptionally activated during differentiation of the stem cell into multiple cell types *in vivo* or *ex vivo*;

- (b) inducing a population of said stem cells to differentiate into the particular cell type; and
- (c) assaying to detect two or more of different genes identified in step (a) that are transcriptionally activated at different times in cells that are differentiating into the particular cell type.

19. The method of claim 18, further comprising determining the nucleotide sequence of at least part of a gene detected in step (c) that is transcriptionally activated when the stem cells differentiate into the particular cell type.

20. The method of claim 18, wherein step (b) comprises assaying to detect a change in the concentration of a mRNA or a polypeptide resulting from transcriptional activation of a gene of the stem cell during differentiation of the stem cell into the particular cell type.

21. The method of claim 18, wherein the stem cell is a totipotent, nearly totipotent, or pluripotent stem cell.

22. The method of claim 18, wherein the stem cell is an embryonic stem cell or embryonic germ cell of a murine, bovine, porcine, non-human primate, or human mammal.

23. The method of claim 18, wherein at least one of the genes detected in step (c) is transcriptionally activated only in cells differentiating into the particular cell type.

24. The method of claim 18, wherein at least one of the genes detected in step (c) is transcriptionally activated in two or more lineages of cells differentiating into different cell types.

25. The method of claim 18, comprising inducing the stem cells to differentiate into a pluripotential stem cell or a progenitor cell committed to giving rise to one or a few fully differentiated cell types.

26. The method of claim 18, comprising inducing the stem cells to differentiate into a fully differentiated cell type.

27. The method of claim 18, comprising inducing the stem cells to differentiate into a particular cell type *in vivo*, and assaying to detect transcriptional activation of two or more genes at different times *in vivo* in cells differentiating into the particular cell type.

28. The method of claim 27, further comprising identifying the location *in vivo* of a cell in which is detected a gene that is transcriptionally activated in cells differentiating into the particular cell type.

29. The method of claim 27, further comprising determining the nucleotide sequence of at least part of a gene that is detected as being transcriptionally activated in cells differentiating into the particular cell type.

30. The method of claim 27, wherein assaying comprises monitoring to detect a change in the concentration of a mRNA encoded by one of the genes.

31. The method of claim 30, comprising monitoring to detect a change in the concentration of a mRNA by *in situ* hybridization with fluorescent hybridization probes (FISH) or radiolabeled hybridization probes.

32. The method of claim 30, comprising using reverse-transcription polymerase chain reaction (RT-PCR) to detect a change in the concentration of a mRNA.

33. The method of claim 18, wherein assaying comprises monitoring to detect a change in the concentration of a protein encoded by one of the genes.

34. The method of claim 33, wherein a protein encoded by one of the genes is an antigenic protein, and

the method comprises using antibodies that bind specifically to the antigenic protein in assaying to detect a change in the concentration of an antigenic protein.

35. The method of claim 27, wherein at least one marker DNA construct is inserted into the genomic DNA of the stem cells and is transcriptionally activated when the stem cells differentiate into the particular cell type; and the method comprises assaying to detect the expression of the marker DNA construct during the time the stem cells are differentiating into the particular cell type.

36. The method of claim 35 wherein the DNA construct is operably linked to an exogenous cell-specific promoter.

37. The method of claim 35 wherein the DNA construct is operably linked to an endogenous promoter.

38. The method of claim 35 wherein the cells are incorporated into a non-human animal and the assay is effected *in vivo*.

39. The method of claim 35, wherein the marker DNA construct is inserted into an endogenous gene and transcription of the marker DNA construct is controlled by the promoter of the endogenous gene.

40. The method of claim 39, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that

confers resistance to a selection agent, an intracellular antigenic protein, and an antigenic cell surface protein that is exposed to the cell exterior.

41. The method of claim 27, wherein the stem cell is an embryonic stem cell, and the method comprises introducing the embryonic stem cell into a host nonhuman mammal in which it forms a teratoma comprising a differentiated cell of said particular cell type.

42. The method of claim 41 wherein said ES cells comprise a marker DNA construct randomly inserted that encodes a detectable product.

43. The method of claim 27 wherein the stem cell is a totipotent or nearly totipotent stem cell of a non-human mammal, the method further comprising:

generating a non-human embryo with the stem cell;

allowing the embryo to develop; and

assaying to detect transcriptional activation of two or more genes at different times in cells of the developing non-human mammal that are differentiating into the particular cell type.

44. The method of claim 43, wherein the stem cell is an embryonic stem cell or embryonic germ cell of a murine, bovine, porcine, or non-human primate mammal.

45. The method of claim 43 wherein said stem cells comprise a DNA encoding a detectable product randomly inserted into the genomic of said cells.

46. The method of claim 41, comprising generating a non-human embryo with the stem cell, in the genomic DNA of which is inserted a marker

DNA construct that is transcriptionally activated in cells that are differentiating into the particular cell type;

wherein the assaying step comprises assaying to detect transcriptional activation of the marker DNA construct in cells that are differentiating into the particular cell type.

47. The method of claim 46 wherein the marker DNA is operably linked to an endogenous promoter or a heterologous promoter that is tissue specific.

48. The method of claim 46, wherein the marker DNA construct is inserted into an endogenous gene and transcription of the marker DNA construct is controlled by the promoter of the endogenous gene.

49. The method of claim 48, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and an antigenic cell surface protein that is exposed to the cell exterior.

50. The method of claim 48, further comprising identifying the location in vivo of a cell having a gene that is transcriptionally activated in cells differentiating into the particular cell type.

51. The method of claim 48, wherein assaying comprises monitoring to detect a change in the concentration of a mRNA by in situ hybridization with fluorescent hybridization probes (FISH) or radiolabeled hybridization probes.

52. The method of claim 48, wherein assaying comprising using reverse-transcription polymerase chain reaction (RT-PCR) to detect a change in the concentration of a mRNA.

53. The method of claim 48, wherein a protein encoded by one of the genes is antigenic, and assaying comprises monitoring to detect a change in the concentration of an antigenic protein using antibodies that bind specifically to the antigenic protein.

54. The method of claim 53, comprising using immunocytochemistry to detect a change in the concentration of the antigenic protein.

55. The method of claim 48, further comprising determining the nucleotide sequence of at least part of a gene in which is inserted a marker DNA construct that is transcriptionally activated in cells that are differentiating into the particular cell type.

56. The method of claim 46, wherein the embryo is produced by nuclear transfer cloning using a nuclear donor cell in the genomic DNA of which is inserted a marker DNA construct that is transcriptionally activated when the stem cell differentiates into a particular cell type.

57. The method of claim 56 wherein the marker DNA operably linked to an endogenous or heterologous tissue specific promoter.

58. The method of claim 46, wherein the embryo is a chimeric embryo that comprises at least one stem cell that does not have said marker DNA construct.

59. The method of claim 58 wherein the marker DNA is operably linked to an endogenous or heterologous tissue specific promoter.

60. The method of claim 18, comprising inducing the stem cells to differentiate into the particular cell type ex vivo, and assaying to detect two or

more genes that are transcriptionally activated at different times in cells of a lineage formed by differentiation of the stem cells into the particular cell type.

61. The method of claim 60, wherein the stem cells are embryonic stem cells or embryonic germ cells of a murine, bovine, porcine, non-human primate, or human mammal, and

the method comprises inducing the stem cells to form an embryoid body comprising a cell that differentiates into said particular cell type.

62. The method of claim 60, wherein step (b) comprises assaying to detect a change in the concentration of a mRNA or a polypeptide resulting from transcriptional activation of a gene of the stem cell during differentiation of the stem cell into the particular cell type.

63. The method of claim 60, wherein the stem cell is a totipotent, nearly totipotent, or pluripotent stem cell of a mammal.

64. The method of claim 60 wherein the cells are isolated or \_\_\_\_\_.

65. The method of claim 60 wherein the cells contain more than one marker gene.

66. The method of claim 60 wherein the sequence of the gene is determined a nucleic acid hybridization probe that hybridizes specifically to said nucleotide sequence of a gene that is transcriptionally activated when stem cells differentiates into a particular differentiated or partially differentiated cell type is used to identify said gene or related genes that are conditionally expressed in a particular cell type.

67. A method for identifying two or more genes that are transcriptionally activated at different times in cells that are differentiating into a particular cell type, comprising:

- (a) obtaining stem cells comprising two or more different endogenous genes, in each of which is inserted a marker DNA construct that is controlled by the promoter of the endogenous gene and is transcriptionally activated when the stem cells differentiate;
- (b) inducing the stem cells to differentiate into the particular cell type;
- (c) monitoring to detect the activation of transcription of marker DNA constructs in the differentiating cells, and screening to identify two or more different genes containing marker DNA constructs that are transcriptionally activated at different times in cells differentiating into the particular cell type.

68. The method of claim 67, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and an antigenic cell surface protein that is exposed to the cell exterior.

69. The method of claim 67, wherein monitoring comprises assaying to detect a change in the concentration of a mRNA or a polypeptide encoded by a marker DNA construct in cells differentiating into the particular cell type.

70. The method of claim 67 wherein the cells contain more than one marker gene.

71. The method of claim 67 wherein the marker gene is operably linked to an endogenous or tissue specific promoter.

72. The method of claim 67, wherein at least one of the genes detected in step (c) is transcriptionally activated only in cells differentiating into the particular cell type.

73. The method of claim 67, wherein at least one of the genes detected in step (c) is transcriptionally activated in two or more lineages of cells differentiating into different cell types

74. The method of claim 67, further comprising determining the nucleotide sequence of at least part of an endogenous gene in which is inserted a marker DNA construct that is transcriptionally activated in cells that are differentiating into the particular cell type.

75. The method of claim 67, comprising inducing the stem cells to differentiate into a pluripotential stem cell or a progenitor cell committed to giving rise to one or a few fully differentiated cell types.

76. The method of claim 67, comprising inducing the stem cells to differentiate into a fully differentiated cell type.

77. The method of claim 71 wherein one marker is recombinant mediated and the other marker is randomly inserted.

78. The method of claim 67, comprising inducing the stem cells to differentiate into the particular cell type in vivo, and monitoring to detect the activation of transcription of marker DNA constructs in cells that are differentiating in vivo into the particular cell type.

79. The method of claim 78, further comprising identifying the location in vivo of a cell containing a marker DNA construct that is transcriptionally activated in cells differentiating into the particular cell type.

80. The method of claim 77, wherein assaying comprises monitoring to detect a change in the concentration of a mRNA encoded by a marker DNA construct by in situ hybridization with fluorescent hybridization probes (FISH) or radiolabeled hybridization probes.

81. The method of claim 77 wherein marker DNA constructs encode an antigenic protein, and assaying comprises monitoring to detect a change in the concentration of the antigenic protein using antibodies that bind specifically to the antigenic protein.

82. The method of claim 81, comprising using immunocytochemistry to detect a change in the concentration of the antigenic protein.

83. The method of claim 77, further comprising determining the nucleotide sequence of at least part of an endogenous gene in which is inserted a marker DNA construct that is transcriptionally activated in cells that are differentiating in vivo into the particular cell type.

84. The method of claim 77, wherein the stem cell is a totipotent or nearly totipotent stem cell of a non-human mammal, the method further comprising:

generating a non-human embryo with the stem cell;

allowing the embryo to develop; and

monitoring to detect the activation of transcription of the marker DNA constructs in cells of the developing non-human mammal that are differentiating into the particular cell type.

85. The method of claim 84, wherein the stem cell is an embryonic stem cell or embryonic germ cell of a murine, bovine, porcine, or non-human primate mammal.

86. The method of claim 84, further comprising identifying the location in vivo of a cell containing a marker DNA construct that is transcriptionally activated in cells differentiating into the particular cell type.

87. The method of claim 84, wherein the embryo is produced by nuclear transfer cloning using a nuclear donor cell in the genomic DNA of which is inserted a marker DNA construct that is transcriptionally activated when the stem cell differentiates into a particular cell type.

88. The method of claim 84, wherein the embryo is a chimeric embryo that comprises at least one stem cell that does not have said marker DNA construct.

89. The method of claim 77, wherein the stem cell is an embryonic stem cell, and the method further comprises:

introducing the embryonic stem cell into a host non-human mammal in which it forms a teratoma comprising a differentiated cell of the particular cell type; and

monitoring to detect the activation of transcription of the marker DNA constructs in cells of the teratoma that are differentiating into the particular cell type.

90. The method of claim 67, further comprising purifying a cell containing a marker DNA construct that is transcriptionally activated in cells

differentiating into the particular cell type by separating the cell from cells in which said marker DNA construct is not transcriptionally activated.

91. The method of claim 90, wherein the marker DNA construct encodes an antigenic cell surface protein, and the cells having the antigenic cell surface protein are purified from cells that do not have the protein by laser dissection, cell sorting, or immunoaffinity separation.

92. The method of claim 90, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, an antigenic cell surface protein that is exposed to the cell exterior, or a fusion protein comprising at least one of the foregoing proteins, and the cells are purified by cell sorting, laser dissection, or immunoaffinity separation.

93. The method of claim 90, wherein the marker DNA construct encodes a protein that confers resistance to a selection agent, and purification of the cells comprises culturing the cells in the presence of an agent that selects against non-resistant cells.

94. The method of claim 67, wherein step (a) comprises obtaining multiple lines of a stem cell, each line having at least one gene in which is inserted a marker DNA construct that is transcriptionally activated when the stem cells differentiate;

and the method further comprises identifying cells of two or more different lines of the stem cell, each having a different gene in which is inserted a marker DNA construct that is transcriptionally activated at a different time in cells differentiating into the particular cell type.

95. The method of claim 94 wherein marker is operably linked to an endogenous or heterologous tissue specific promoter.

96. The method of claim 68, wherein step (a) comprises obtaining multiple lines of a stem cell, each line having two or more genes in which are inserted marker DNA constructs that are transcriptionally activated when the stem cells differentiate;

and the method further comprises identifying at least one stem cell line having two or more genes in which are inserted marker DNA constructs that are transcriptionally activated at different times in cells differentiating into the particular cell type.

97. The method of claim 1, wherein  
step (a) comprises randomly inserting into at least two different sites in the genomic DNA of each stem cell in a population of stem cells,  
wherein each inserted marker DNA construct comprises a nucleotide sequence that encodes the same or a different detectable product, at least one of which nucleotide sequences is not operably linked to a promoter; and  
step (c) comprises monitoring the differentiating stem cells to detect changes in the level of expression of both marker DNA constructs in each stem cell.

98. The method of claim 97, wherein at least two marker DNA constructs inserted in the genomic DNA of each stem cell encode different detectable products.

99. The method of claim 97, wherein at least one marker DNA construct in each stem cell comprises an exogenous promoter that is operably linked to a

nucleotide sequence that encodes a detectable product and is transcriptionally activated in cells that are differentiating into a particular cell type.

100. The method of claim 97, further comprising identifying individual stem cells having at least two marker DNA constructs that are transcriptionally activated or repressed when the stem cells differentiate, and isolating and expanding said stem cells as single cell clones.

101. The method of claim 1,

wherein the marker DNA construct encodes a recombinase,

wherein the same or a different marker DNA construct inserted into the genomic DNA of each stem cell comprises a first nucleotide sequence encoding a detectable product, and a second nucleotide sequence comprising two recombination sites that inhibits the expression of the first nucleotide sequence;

wherein the recombinase recognizes the two recombination sites, and expression of the nucleotide sequence encoding recombinase results in excision of the second nucleotide sequence from the stem cell genomic DNA and expression of the first nucleotide sequence encoding the detectable product; and

wherein step (d) comprises determining the relative timing of the transcriptional activation of the marker DNA constructs that occurs when the stem cells differentiate.

102. The method of claim 1,

wherein the marker DNA construct encodes a recombinase,

wherein the same or a different marker DNA construct inserted into the genomic DNA of each stem cell comprises:

(i) a first nucleotide sequence encoding a detectable product, and

(ii) a second nucleotide sequence comprising two recombination sites that inhibits the expression of the first nucleotide sequence;

wherein the recombinase recognizes the two recombination sites, and expression of the nucleotide sequence encoding recombinase results in excision of the second nucleotide sequence from the stem cell genomic DNA and expression of the first nucleotide sequence encoding the detectable product.

103. A composition comprising isolated stem cells comprising two or more marker DNA constructs that are transcriptionally activated at different times during differentiation of the stem cells into a particular cell type.

104. The composition of claim 103 wherein the marker genes are operably linked to tissue specific heterologous promoters.

105. The composition of claim 103, wherein at least one marker DNA construct is inserted into an endogenous gene and its transcription is controlled by the endogenous promoter of the gene in which it is inserted.

106. The composition of claim 103, wherein transcription of at least one of the marker DNA constructs is controlled by an exogenous promoter that is a cell type-specific or differentiation stage-specific promoter.

107. The composition of claim 103 comprising cells of two or more different lines of said stem cell, the cells of each line having at least one gene in which is inserted a marker DNA construct that is transcriptionally activated at a different time interval during differentiation of the stem cells into the particular cell type.

108. The composition of claim 103 comprising at least one stem cell having two or more different genes, in each of which is inserted a marker DNA construct that is transcriptionally activated at a different time during differentiation of the stem cell into the particular cell type.

109. The composition of claim 103, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and an antigenic cell surface protein that is exposed to the cell exterior.

110. The composition of claim 103, wherein the stem cell is an embryonic stem cell or embryonic germ cell of a murine, bovine, porcine, non-human primate, or human mammal.

111. A method for determining conditions that induce stem cells to differentiate or partially differentiate in vitro into a particular cell type, comprising,

- (a) obtaining stem cells having genes in which a marker DNA construct is randomly inserted,
- (b) screening to identify stem cells comprising two or more different genes in which are inserted marker DNA constructs that are transcriptionally activated at different times in cells of the lineage formed by differentiation of the stem cells into the particular cell type;
- (c) culturing the stem cells identified in (b) in the presence of one or more combinations of chemical, biological, and physical agents that are putatively differentiation-inducing, and assaying to identify one or more

combinations of conditions in which the two or more genes are transcriptionally activated; and

(d) culturing the stem cells under the conditions identified in step (c) to induce the stem cells to differentiate or partially differentiate into the particular cell type.

112. The method of claim 111, further comprising isolating the stem cells identified in step (b), and expanding the isolated stem cells as single cell clones.

113. The method of claim 111, wherein the stem cells are totipotent or nearly totipotent stem cells.

114. The method of claim 111, wherein the stem cells are embryonic stem cells or embryonic germ cells of a murine, bovine, porcine, non-human primate, or human mammal.

115. The method of claim 111, wherein the marker DNA construct encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, a protein that confers resistance to a selection agent, an intracellular antigenic protein, and an antigenic cell surface protein that is exposed to the cell exterior.

116. The method of claim 111, further comprising determining the nucleotide sequence of at least part of the gene in which is inserted a marker DNA construct that is transcriptionally activated when the stem cell differentiates.

117. The method of claim 111, wherein step (c) comprises assaying to detect a change in the concentration of a mRNA resulting from transcriptional

activation of a gene in a cell of the lineage formed by differentiation of said stem cell into said particular cell type.

118. The method of claim 117, comprising assaying to detect a change in the concentration of a mRNA by in situ hybridization with fluorescent hybridization probes (FISH) or radiolabeled hybridization probes.

119. The method of claim 118, comprising using reverse-transcription polymerase chain reaction (RT-PCR) to detect a change in the concentration of a mRNA.

120. The method of claim 111, wherein step (c) comprises assaying to detect a change in the concentration of a protein that is synthesized during differentiation of said stem cell into a particular cell type.

121. The method of claim 118, comprising assaying to detect a change in the concentration of a surface protein comprising an antigenic portion that is exposed to the cell exterior.

122. The method of claim 111, wherein at least one of said two or more genes identified in step (b) is a uninodal locus that is only transcriptionally activated during differentiation of the stem cell into the particular cell type.

123. The method of claim 111, wherein at least one of said two or more genes identified in step (b) is a multinodal locus that is transcriptionally activated when the stem cell differentiates into at least two different cell types.

124. A method for inducing stem cells to differentiate or partially differentiate in vitro into a particular cell type, comprising,

- (a) identifying a first gene that is transcriptionally activated in a cell of the lineage formed by differentiation of the stem cells into the particular cell type;
- (b) identifying a second gene that is transcriptionally activated after the first gene in a cell of the lineage formed by differentiation of the stem cells into the particular cell type;
- (c) culturing the stem cells in the presence of at least one combination of chemical, biological, and physical agents in which the first gene is transcriptionally activated,
- (d) purifying the partially differentiated cells by separating cells in which the first gene is transcriptionally activated from cells in which it is not transcriptionally activated; and
- (e) culturing the stem cells in the presence of at least one combination of chemical, biological, and physical agents in which the second gene is transcriptionally activated, to induce the stem cells to further differentiate or partially or differentiate into the particular cell type.

125. The method of claim 124, wherein the second gene is transcriptionally activated in cells that are differentiated into the particular cell type.

126. The method of claim 124, wherein the combination of chemical, biological, and physical agents in step (c) is different from the combination of chemical, biological, and physical agents in step (e).

127. The method of claim 124, wherein the first gene encodes an antigenic cell surface protein that is exposed to the cell exterior, and the cells having the antigenic cell surface protein are purified from cells that do not have the protein.

128. A composition comprising two stem cell lines, each having a separate marker of differentiation

129. A method for identifying cell-cell interactions that induce differentiation, comprising screening sets of at least

two cell lines, each having a separate marker of differentiation, in order to identify cell pairs that interact based on the expression of a differentiated marker.

130. A method for inducing stem cells to differentiate or partially differentiate in vitro into a particular cell type, comprising,

(a) identifying two or more genes that are transcriptionally activated at different times in a cell of the lineage formed by differentiation of the stem cells into the particular cell type;

(b) culturing the stem cells in the presence of one or more combinations of chemical, biological, and physical agents in which the two or more genes identified in step (a) are sequentially transcriptionally activated, to induce the stem cells to differentiate or partially differentiate into the particular cell type.

131. The method of claim 130, wherein step (a) comprises obtaining multiple lines of a stem cell, each line having at least one gene in which is inserted a marker DNA construct that is transcriptionally activated when the stem cells differentiate; and

identifying cells of two or more different lines of the stem cell, each having a different gene in which is inserted a marker DNA construct that is transcriptionally activated at a different time in a cell of a lineage formed by differentiation of the stem cell into the particular cell type;

and step (b) comprises culturing the stem cells identified in step (a) in the presence of the differentiation-inducing agents to induce the stem cells to differentiate or partially differentiate into the particular cell type.

132. The method of claim 130, wherein step (a) comprises obtaining multiple lines of a stem cell, each line having two or more genes in which are inserted marker DNA constructs that are transcriptionally activated when the stem cells differentiate;

identifying at least one stem cell line having two or more genes in which are inserted marker DNA constructs that are transcriptionally activated at different times in a cell of a lineage formed by differentiation of the stem cell into the particular cell type;

and step (b) comprises comprises culturing the stem cells identified in step (a) in the presence of the differentiation-inducing agents to induce the stem cells to differentiate or partially differentiate into the particular cell type.

133. The method of claim 130, wherein the stem cell is a totipotent or nearly totipotent stem cell.

134. The method of claim 130, wherein the stem cell is an embryonic stem cell or embryonic germ cell of a murine, bovine, porcine, non-human primate, or human mammal.

135. The method of claim 130, further comprising purifying the differentiated or partially differentiated cells by separating cells in which at least one of the genes identified in step (a) is transcriptionally activated from cells in which said locus is not transcriptionally activated.

136. The method of claim 129, wherein at least one of the genes identified in step (a) encodes an antigenic cell surface protein that is exposed to the cell exterior, and the cells having the antigenic cell surface protein are purified from cells that do not have the protein.

137. The method of claim 130, wherein the cells having the antigenic cell surface protein are purified from cells that do not have the protein by laser dissection, cell sorting, or immunoaffinity separation.

138. The method of claim 135,

wherein the stem cells have inserted into their genome a marker DNA construct comprising a nucleotide sequence encoding a polypeptide that is transcriptionally activated when at least one of the genes identified in step (a) is transcriptionally activated,

and the differentiated or partially differentiated cells are purified by separating cells in which the marker DNA construct is expressed from cells in which it is not expressed.

139. The method of claim 138, wherein the marker DNA construct comprises a nucleotide sequence that encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, an antigenic cell surface protein that is exposed to the cell exterior, or a fusion protein comprising at least one of the

foregoing proteins, and the cells are purified by cell sorting, laser dissection, or immunoaffinity separation.

140. The method of claim 138, wherein the marker DNA construct comprises a nucleotide sequence encoding a protein that confers resistance to a selection agent, and purification of the cells comprises culturing the cells in the presence of an agent that selects against non-resistant cells.

141. The method of claim 138, wherein the marker DNA construct comprises at least two nucleotide sequences that each encode a polypeptide and are expressed in a cell of the lineage formed by differentiation of the stem cell into the particular cell type.

142. The method of claim 138, wherein the marker DNA construct comprises a nucleotide sequence that is operably linked to promoter that is of the same or different species as the stem cells, and is a constitutive promoter, an inducible promoter, a developmental stage-specific promoter, or a cell type-specific promoter.

143. The method of claim 138,  
wherein a marker DNA construct is inserted into at least one of the genes identified in step (a) and is transcriptionally activated when the locus in which it is inserted is transcriptionally activated,

and the differentiated or partially differentiated cells are purified by separating cells in which the marker DNA construct is expressed from cells in which it is not expressed.

144. The method of claim 143, wherein insertion of the marker DNA construct into a gene identified in step (a) partially or completely inhibits the expression of the protein encoded by the gene.

145. The method of claim 143, wherein insertion of the marker DNA construct into a gene identified in step (a) of a stem cell does not inhibit the expression of the protein encoded by the gene.

146. The method of claim 138,

wherein the marker DNA construct comprises a nucleotide sequence encoding a detectable product operably linked to an conditional promoter that activates transcription of the marker DNA at approximately the same time that at least one of the genes identified in step (a) is transcriptionally activated;

and the partially or completely differentiated cells are purified by separating cells in which the marker DNA construct is expressed from cells in which it is not expressed.

147. The method of claim 146, wherein the conditional promoter is an inducible promoter, a developmental stage-specific promoter, or a cell type-specific promoter, and is of the same or different species as the stem cells.

148. The method of claim 138,

wherein the marker DNA construct is inserted into at least one of the genes identified in step (a) and comprises a nucleotide sequence encoding a recombinase that is transcriptionally activated when the locus in which it is inserted is transcriptionally activated;

and the same or a different marker DNA construct inserted in the genome of the stem cells also comprises a first nucleotide sequence encoding a detectable

product, and a second nucleotide sequence comprising two recombination sites that inhibits the expression of the first nucleotide sequence; and

wherein the recombinase recognizes the two recombination sites, and expression of the nucleotide sequence encoding recombinase results in excision of the second nucleotide sequence from the stem cell genomic DNA and expression of the first nucleotide sequence encoding the detectable product.

149. The method of claim 138,

wherein the marker DNA construct comprises a nucleotide sequence encoding a detectable product, and further comprises two recombination sites flanking at least one nucleotide sequence that is required for expression of the first DNA;

and the same or a different marker DNA construct inserted in the genome of the stem cells comprises a conditional promoter that is operably linked to an nucleotide sequence encoding a recombinase that recognizes the two recombination sites; and

wherein activation of transcription of the nucleotide sequence encoding the recombinase results in excision of the nucleotide sequence required for expression of the DNA encoding the detectable product, thereby inhibiting expression of the DNA encoding the detectable product.

150. The method of claim 149, wherein the conditional promoter is of the same or different species as the stem cells, and is a constitutive promoter, an inducible promoter, a developmental stage-specific promoter, or a cell type-specific promoter.

151. A method for producing an antibody that binds specifically to a differentiation antigen of a particular differentiated or partially differentiated cell type, comprising,

- (a) obtaining a stem cell having inserted in its genomic DNA a marker DNA construct comprising a nucleotide sequence encoding a detectable product that is transcriptionally activated in cells of a particular differentiated or partially differentiated cell type,
- (b) inducing the stem cells to differentiate into the particular differentiated or partially differentiated cell type;
- (c) monitoring the differentiating stem cells to detect activation of transcription of the marker DNA construct in the cells;
- (d) purifying cells having a transcriptionally activated marker DNA construct; and
- (e) using the purified cells or an extract thereof as an immunogen to elicit production of an antibody that binds specifically to a differentiation antigen of the purified cells.

152. The method of claim 151, wherein step (b) comprises culturing the cells ex vivo under conditions in which the stem cells differentiate into the particular differentiated or partially differentiated cell type.

153. The method of claim 151, wherein step (e) comprises eliciting production of an antibody that binds specifically to a differentiation antigen on the external surface of the cells.

154. The method of claim 153, comprising eliciting production of a monoclonal antibody that binds specifically to a differentiation antigen on the external surface of the cells.

155. The method of claim 153, comprising eliciting production of polyclonal antibodies that bind specifically to a differentiation antigen on the external surface of the cells.

156. The method of claim 152, wherein the marker DNA construct comprises a nucleotide sequence that encodes a protein selected from the group consisting of a fluorescent protein, an enzyme that catalyzes production of a chromogenic or fluorescent product, an antigenic cell surface protein that is exposed to the cell exterior, or a fusion protein comprising at least one of the foregoing proteins, and the cells are purified by cell sorting, laser dissection, or immunoaffinity separation.

157. The method of claim 152, wherein the marker DNA construct comprises a nucleotide sequence encoding a protein that confers resistance to a selection agent, and purification of the cells comprises culturing the cells in the presence of an agent that selects against non-resistant cells.